

Rational design of freeze-drying formulations: a Molecular Dynamics approach

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# Rational design of freeze-drying formulations: A Molecular Dynamics approach

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## PURPOSE

Even though freeze-drying is widely used for the preparation of biopharmaceuticals, it causes stresses which can result in unfolding or aggregation. Suitable excipients must therefore be added to avoid loss of activity. However, at present, the choice of a suitable formulation is mainly empirical, due to a lack of knowledge about the phenomena involved. In this context, the objective of the present work was to understand the molecular mechanisms at the basis of protein stabilization, and to guide the choice of suitable excipients.

## METHODS

To reach this goal, Molecular Dynamics (MD) was used, and a model protein, the human growth hormone (hGH), was simulated during freezing or drying, both in the presence and absence of excipients. Enhanced sampling techniques, such as Metadynamics, were also employed to overcome the time limitations of classical MD.

## RESULTS

The unfolding pathway was calculated, and we found that both freezing and drying stresses promoted the formation of an aggregation-prone intermediate of hGH [1]. However, this unfolding process was inhibited in the presence of excipients, especially the disaccharides. We found that sucrose and lactose provided the best thermodynamic stabilization, because of their high preferential exclusion from the protein surface, especially during the freezing step. However, trehalose and cellobiose were much more performant during drying, because of their ability to form a very viscous and highly ordered matrix, capable of providing kinetic stabilization. Moreover, we also discovered [2] that surfactants can act as molecular chaperones and foster protein refolding, thus stabilizing proteins against aggregation.

## CONCLUSION

Our study has demonstrated that MD can allow the identification of the molecular properties at the basis of protein stabilization, thus simplifying the choice of a suitable formulation.

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